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Original Article

Long-term daily high and low doses of azithromycin in children with cystic fibrosis: A randomized controlled trial

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Abstract

Background: Long-term administration of azithromycin (AZM) in children with cystic fibrosis (CF) has improved outcomes. However, the doses and schedule of administration are not very well studied in children with CF.
Methods: A randomized controlled trial was conducted to compare the effect of two doses of azithromycin (5 mg/kg/day and 15 mg/kg/day) on FEV_1 and pulmonary exacerbations in children with cystic fibrosis. Enrolled children were randomly allocated to receive daily azithromycin (5 mg/kg/day or 15 mg/kg/day) for 6 months. Clinical assessment and FEV_1 measurement were performed monthly.
Results: 56 children (28 in high dose group and 28 in low dose group) were enrolled. 47 (24 and 23 children in low and high dose groups) completed 12 months of follow up. There was no difference in clinical scores, FEV_1, pulmonary exacerbation rates between two groups at baseline, 6 months and at 12 months. Per protocol analysis revealed that pulmonary exacerbation increased after discontinuing AZM and there was significantly more increase after 12 months of enrolment in children getting high dose azithromycin. There was no improvement in FEV_1 in either group at the end of treatment period. Children tolerated daily low as well as high dose AZM well for 6 months. There was no significant side effect of azithromycin.
Conclusion: In this randomized controlled trial, we did not find differences in the effect of 2 doses (5 mg/kg/day or 15 mg/kg/day) of AZM on change in percentage predicted FEV_1, clinical scores, *Pseudomonas* colonization rates, pulmonary exacerbations and need for antibiotics. There was increase in exacerbations after stopping azithromycin in both the groups. Our results also suggest that the decrease in the incidence of LRTI persists only till 6 months after discontinuing azithromycin.

Keywords: Azithromycin; Clinical scores; Cystic fibrosis; FEV_1

1. Introduction

Cystic fibrosis (CF) is a multisystem disorder and pulmonary involvement is most dramatic. Patients with cystic fibrosis are characterized by recurrent respiratory infection and inflammation along with fat malabsorption and micronutrient deficiency. Pulmonary manifestations are because of infection with *Pseudomonas* and early and relentless pulmonary inflammation [1–3]. There is growing interest in the use of macrolides as immune modulating drugs in cystic fibrosis. They have been shown to decrease sputum viscoelasticity and airway adhesion of *Pseudomonas aeruginosa* [4].

There are studies to document improved outcome in children with CF treated with azithromycin (AZM) as compared to placebo [5–8]. Despite the availability of this new information, many questions remain unanswered about the use of AZM in cystic fibrosis including the dose, the correct dose interval, its duration of effect and the impact of long-term treatment with AZM on disease progression and the microbiology of the lung. Daily versus once a week administration of AZM has been compared and shown equivalent results [9] but there are no studies to compare different doses of AZM in children. We conducted a study with the hypothesis that children with cystic fibrosis treated with high dose azithromycin (15 mg/kg) once

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daily for 6 months will have greater than 10% change in percent predicted FEV_1 as compared to patients treated with azithromycin (5 mg/kg) once daily for 6 months.

2. Methods

This randomized controlled trial was conducted in the Pediatric Chest Clinic of All India Institute of Medical Sciences, New Delhi India. Children with cystic fibrosis of either sex, 5–18 years of age being followed up in the clinic were subjects of study. Patients were diagnosed with cystic fibrosis on the basis of characteristic signs and symptoms, plus a positive sweat test at least twice, or two defined cystic fibrosis gene mutations.

2.1. Inclusion criteria

Children between 5 years and 18 years diagnosed as cystic fibrosis as defined above who were able to perform spirometry, able to swallow suspension, willing to come for follow up, had forced expiratory volume (FEV_1) of less than 80% predicted, clinically stable at the time of enrolment and no intravenous antibiotics administered for at least 2 weeks prior to enrolment, were eligible for the study.

2.2. Exclusion criteria

Children with cystic fibrosis related liver disease, or liver function tests greater than 3 times the laboratory upper limit, who had received macrolide antibiotics or oral steroids for more than 7 days in the month prior to enrolment, with history of allergy to macrolides or known or suspected intolerance to sputum induction, were excluded.

2.3. Methods

Parents of children who fulfilled inclusion criteria and did not have exclusion criteria were invited to participate in the study. A written informed consent was obtained from parents. Children were randomized to receive 5 mg/kg/day or 15 mg/kg/day of azithromycin by computer generated blocks of four. Children were given azithromycin in addition to supportive care for the first 6 months. Supportive care included: regular physiotherapy (postural drainage), enzyme replacement, vitamins A, D and E supplementation (2 RDA), liberal salt intake, inhaled bronchodilators and steroids and antibiotics whenever detected to have pulmonary exacerbation. Children were assessed to have pulmonary exacerbation if there was increase frequency, severity or duration of cough with change in amount or nature of expectoration. Children with pulmonary exacerbations were hospitalized if there was breathlessness (increased respiratory rates with chest indrawing) and/or hypoxia (oxygen saturation <90%). They were treated with intravenous ceftazadime and amikacin (or other antibiotics depending on isolation and sensitivity of pathogens) for 2 weeks. Children with pulmonary exacerbation but no breathlessness or hypoxia were treated on ambulatory basis with fluoroquinolones if colonized with *Pseudomonas* or amoxy-clavulanic acid if not colonized with *Pseudomonas*. Antibiotics were changed according to culture reports. None of the study subjects during study period received alfa dornase (DNASe) or inhaled tobramycin.

Patients were followed up every month for a minimum of 12 months. Azithromycin suspension was dispensed with adequate supply for 1 month on each follow up visit for the first 6 months. At every visit a history and physical examination was performed. The patient's weight was measured to the nearest 0.1 kg, and the height in centimeter to the nearest mm using a stadiometer. Spirometry was performed according to the American Thoracic Society/ERS guidelines [10] and sputum (children above 6 years of age) or cough swab (children below 6 years of age) cultures [11] was obtained. All changes in symptoms and treatment were recorded on the predesigned performa. Any need for oral or intravenous antibiotics or hospitalization was recorded.

On each visit patients were assessed on modified clinical scores that are being used routinely in our clinic [12]. All children were assessed on scores for general activity, physical examination and nutritional status. Each category scored from 5 to 25, maximum scores of 75 indicating excellent clinical condition. Patient were assessed to be having mild illness, moderate illness and severe illness on basis of clinical scores of 57–75, 38–57 and <37 respectively.

Before the treatment period began blood was drawn from each patient for ESR and liver function tests. Sputum/cough swab was collected for microbiological analysis on each visit.

2.4. Sputum microbiology

Sputum was cultured by using standard techniques [13]. We studied the effect of azithromycin on production of mucoidy by *P. aeruginosa*. Children with at least two positive cultures for *Pseudomonas* in past 12 months were considered as colonized with *Pseudomonas*. Other organisms on culture with sensitivity were recorded.

2.5. Adherence to the medications

Adherence to azithromycin was assessed by asking the parents and seeing the medications that remained unused on each visit.

2.6. Sample size

The primary study outcome was change in FEV_1 % predicted and was based on improvements in the FEV_1 reported in the open study by Jaffé et al. [14]. A sample size of 8 per group is required to detect a difference of 10% between high and low doses of AZM with 90% power and 5% statistical significance.

2.7. IL-8 estimation

The concentrations of IL-8 were determined by quantitative sandwich enzyme immunoassay technique, using a IL-8 ELISA

IM 2237 (Cell Com, Beckman Coulter, France) kit as per the manufacturer's instructions. Standard curve of IL-8 was plotted using different concentrations of IL-8 standard — 0, 31.2, 125, 500, and 2000 pg/mL. For estimation of IL-8 concentration in a sputum sample, the sol phase was prepared [15]. The samples were stored at −70 °C till they were processed. The O.D. was measured at 450 nm with a microplate reader (Thermo Electron Corporation, Multiskan EX).

2.8. Statistical tests

Baseline characteristics were compared between the two groups. Differences in FEV_1 between the 2 groups were assessed by the Student's *T* test and differences in secondary outcomes by the chi square test. STATA (College station, Texas, USA) was used for statistical analysis.

The study was approved by the Ethics committee of our institution.

3. Results

The study was conducted from August 2005 to Dec 2007. A total of 56 (28 patients in each group) children with cystic fibrosis were enrolled. Nine patients (4 in low dose group and 5 in high dose group) did not complete follow up of 12 months. For final analysis, 24 children received azithromycin 5 mg/kg/day (group A) and 23 received 15 mg/kg/day (group B) for 6 months. Some children (8 in low dose group and 9 in high dose group) were followed up to 18 months (Fig. 1).

3.1. Baseline characteristics

The baseline data of two groups are given in Table 1. They were similar for age, age of onset, clinical features, weight, height, FEV_1, clinical scores, colonization with *Pseudomonas* and common mutations (Delta *F* 508). There were more boys in the group receiving high dose AZM (15 mg/kg/day).

Table 1
Baseline characteristics of patients in two groups.

Characteristics	Group A (*N*=24) (AZM 5 mg/kg/day)	Group B (*N*=23) (AZM 15 mg/kg/day)	*p*
Age mean±SD (years)	9±5.1	7±4.6	0.16
Age at diagnosis mean±SD (years)	5.4±3.6	2.7±2.4	0.004
Sex			
Males	14	20	0.028
Females	10	3	
Clinical features at time of enrolment			
Cough			
Wheeze	21	21	0.672
Breathlessness	8	11	0.32
Recurrent pneumonia	15	16	0.609
Pain chest	5	9	0.179
Hemoptysis	3	3	0.955
Pain abdomen	3	0	0.08
Large bulky stools	5	4	0.764
Oil in stools	8	6	0.587
Mean weight (SD), kg	1	3	0.276
Median height (SD), cm	13.91 (7.17)	14.39 (11.07)	0.569
FEV_1 L (SD)	95.79 (25.02)	95.43 (27.01)	0.481
% predicted FEV_1	1.10 (0.67)	1.09 (0.32)	0.489
	51.07±23.5	65.66±14.80	0.96
Delta *F* 508 homozygous	2	2	1.0
Heterozygous	2	2	
Othr mutations than Delta *F* 508	20	19	
Clinical scores			
Mean (SD)	54.75 (12.08)	53.63 (11.03)	0.378
Chronic *Pseudomonas* infection	10	12	0.47

3.2. Change in percentage predicted FEV_1 and other spirometric parameters

The average percentage predicted FEV_1 did not differ in two groups at baseline, 3 months, 6 months and 12 months between two groups (Table 2). At 18 months the numbers of children in group A and B were 8 and 9 respectively. The percentage predicted FEV_1 was maintained till 9 months after that there

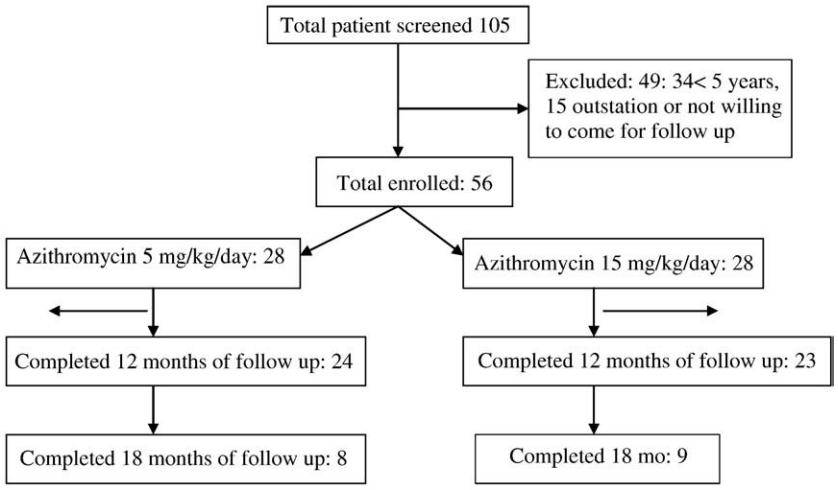

Fig. 1. Trial profile.

was a trend towards decline in FEV_1. In group A the mean values of percentage predicted FEV_1 at time of enrolment at 6 months, 9 months and 12 months were 51, 47, 51 and 52% respectively suggesting no improvement in FEV_1. However, there was a trend showing decline at 12 and 18 months compared to baseline. In group B the FEV_1 values at enrolment, 6 months, 9 months and 12 months were 65, 65, 53 and 57% respectively. There was significant decline at 9, 12 and 18 months. A subgroup analysis was done for 22 children who were chronically infected with *Pseudomonas* (10 and 12 in low and high dose groups). Their baseline characteristics were comparable (data not shown). Their FEV_1 did not differ and the values at baseline were 56.8±20.6 L and 60.6±20.8 L, at 6 months were 57.2±22.00 L and 62.6±25.9 L and at 12 months were 50.6±20.4 L and 55.5±14.9 L.

The other spirometric parameters (FVC, FEF_{25-75} and PEFR) were comparable in two groups at baseline, 6 months, and 12 months (not shown).

3.3. Pulmonary exacerbations and antibiotic use

The numbers of pulmonary exacerbations in two groups were comparable. The rates per child per month during treatment period (first 6 months) were 0.1 and 0.05, between 7 and 12 months 0.2 and 0.17 per child per month and between 13 and 18 months 0.233 and 0.339 in low and high dose groups respectively ($p>0.5$). However, the exacerbations increased significantly during the last 6 months of follow up in high dose group. In low dose group it increased from 0.1 to 0.233 per child per month and in high dose group the rate of pulmonary exacerbations increased from 0.05 to 0.339 per child per month ($p<0.05$). (Table 3).

3.4. Culture of deep throat swabs for Pseudomonas and its sensitivity pattern

The number of children positive for *Pseudomonas* on deep throat swabs in the last 12 months was similar in two groups at different times. A total of 22 (10 in group A, 12 in group B) were positive for *Pseudomonas* in the last 12 months. Two each in two groups were assessed to be producing mucoidy colony of *P. aeruginosa*. All the isolates were resistant to azithromycin as the minimum inhibitory concentration was >8 μg/dL.

A total of 113 pulmonary exacerbations (48 in low dose and 65 in high dose group) were recorded during study period and follow up. Organisms identified on sputum/cough swabs were *P. aeruginosa*, *Staphylococcus aureus*, *Streptococcus hemolyticus*, *Streptococcus pneumoniae* and *Candida albicans* (Table 4). The distribution was similar in two groups.

S. aureus were isolated from 10 samples (4 in low dose and 6 in high dose group) from 6 patients. All were methicillin sensitive.

Table 2
Changes in percentage predicted FEV_1 in relation to time.

Time	Group A Mean (SD) (AZM 5 mg/kg/day)	Group B Mean (SD) (AZM 15 mg/kg/day)	*p*
Baseline	51.07±23.5	65.66±14.80	0.9603
3 months	55.62±23.68	62.62±19.22	0.7366
6 months	47.12±20.00	65.67±29.97	0.9233
9 months	51.5±28.62	53±16.99	0.5344
12 months	52.66±15.04	57.5±12.09	0.6923
18 months	47.5±19.39	45.33±14.57	0.4354
	p comparing Baseline with 6 months: 0.2712	*p* comparing Baseline with 6 months: 0.5006	
	Baseline with 9 months: 0.5221	Baseline with 9 months: 0.0042	
	Baseline with 12 months: 0.6081	Baseline with 12 months: 0.0210	
	Baseline with 18 months: 0.2885	Baseline with 18 months: 0.000	

Table 3
Pulmonary exacerbation rates in two groups.

Time after enrolment	Group A (AZM 5 mg/kg/day)	Group B (AZM 15 mg/kg/day)	*p*
0–6 months	0.1±0.301 per child/month	0.05±0.229 per child/month	0.526
6–12 months	0.2±0.405 per child/month	0.17±0.429 per child/month	0.812
12–18 months	0.24±0.426 per child/month	0.34±0.477 per child/month	0.425
	p comparing exacerbations between the first 6 months with the last 6 months: 0.321	*p* comparing exacerbations between the first 6 months with the last 6 months: 0.0275	

Table 4
Organisms identified in pulmonary exacerbations in two groups.

Microorganisms	Group A (total exacerbations 62) (AZM 5 mg/kg/day) Episodes/patients	Group B (total exacerbations 68) (AZM 15 mg/kg/day) Episodes/patients	*p*
Pseudomonas aeruginosa			
0–6 months	9/7	10/9	
7–12 months	9/6	13/8	
13–18 months	4/4	7/3	
Total	22/12	30/10	0.76
Staphylococcus aureus			
0–6 months	3/2	5/3	
7–12 months	1/1	1/1	
13–18 months	0	0	
Total	4/3	6/3	
			0.60
Streptococcus hemolyticus	2/1	0	–
Streptococcus pneumoniae	0	1/1	–
Candida albicans	1/1	1/1	–
No organisms isolated	33	30	0.43

3.5. Clinical scores

The average composite clinical scores at baseline at different time periods were comparable in two groups (Fig. 2). The average clinical scores in group A at baseline, 6 months, and 12 months were 54, 56 and 47 respectively. A significant trend of improvement till 6 months and subsequent decline at 12 months was observed ($p<0.05$). The average clinical scores at baseline, 6 months and 12 months in group B were 53, 55 and 56 respectively. There was a trend of decline in clinical scores in both the groups at 18 months.

3.6. Changes in the weight of children at different time intervals

The average weights in group A at baseline, 6 months and 12 months were comparable in two groups. The average weights in group A at baseline, 6 months and 12 months were 17, 21 and 17 kg respectively. In group B the average weights at baseline, 6 months and 12 months were 16, 19, and 19 respectively. There was a trend to suggest that average weight increased in the first 6 months after that there was a decline in weight. In group B, there was weight gain for 6 months and after that it was maintained (Table 5).

3.7. IL-8 concentration in sputum

IL-8 levels were performed at baseline and at the end of 6 months in 7 and 6 patients in low and high doses of AZM groups respectively. The levels were more than 2000 pg/mL in all.

3.8. Adherence to the study drug

All children who completed the follow up received >80% of the doses of azithromycin.

4. Discussion

In this randomized controlled trial we did not find difference in the effect of doses of 5 mg/kg/day or 15 mg/kg/day of AZM on change in percentage predicted FEV_1, clinical scores, *Pseudomonas* colonization rates, pulmonary exacerbations and need for antibiotics. There was an increase in exacerbations after stopping azithromycin in both the groups. Though there was no significant improvement in FEV_1 during treatment with azithromycin but there was a significant decline after 3 months of stopping azithromycin.

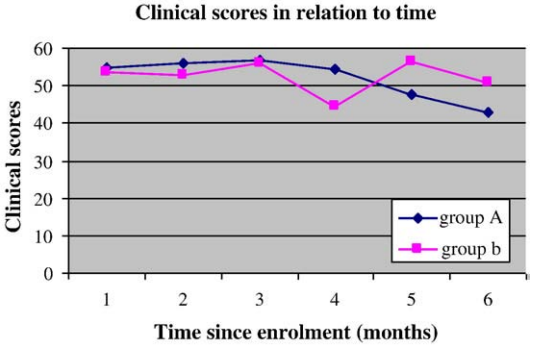

Fig. 2. Clinical scores in two groups with time.

Information on the doses and schedule of AZM treatment in children with CF is scanty. In an earlier report comparing high and low doses of azithromycin (250 mg daily versus twice weekly) in CF patients colonized with *Pseudomonas* it was reported that the sputum concentration of AZM was less in low dose as compared to high dose {9.5 μg/mL (0.6 to 79.3 μg/mL, interquartiles 1.4 to 33.4 μg/mL} and 0.5 μg/mL {range less than 0.1 (below detection level to 5.2 μg/mL, interquartiles 0.2 to 1.4 μg/mL}, suggesting that there may be some effect of different doses on the clinical outcome [16]. The regular doses recommended for azithromycin is 10 mg/kg/day for infections. We decided to compare 5 and 15 mg/kg/day.

There are few studies comparing different doses of azithromycin for comparison with results of our studies. McCormack et al., in their study on 208 patients treated either with 250 mg daily ($n=103$) or 1200 mg weekly ($n=105$) of azithromycin for 6 months, demonstrated equivalence of the two groups with respect to improvements in lung function (forced expiratory volume in one second and forced vital capacity), C-reactive protein, days spent in hospital, admission rates and nutrition. In patients aged <18 years, the daily group had significantly better improvements in *z*-scores for height and weight after 6 months [9]. The dose of daily AZM was 1850 mg per week as compared to 1200 mg in those who received weekly AZM.

Saiman et al. evaluated efficacy of AZM in low dose [250 mg (weight <40 kg) or 500 mg (weight ≥40 kg) of oral azithromycin 3 days a week] for 168 days and demonstrated a significant improvement in FEV_1 at day 168 compared with placebo. They also demonstrated that azithromycin group had less risk of experiencing an exacerbation than participants in the placebo group [7], suggesting efficacy of low dose AZT.

In our study, we did not find significant improvement in spirometric parameters from baseline to end of treatment (at 6 months). The reported improvements in FEV_1 and other responses have been variable. Wolter et al., in their study comparing placebo and azithromycin, documented that in AZM group FEV_1 was maintained but in placebo group there was a decline in the parameters [5]. We did not have a placebo group in our study [5]. Equi et al. in their placebo-controlled crossover trial on 41 CF patients demonstrated that FEV_1 improved by more than 13% in 13 of 41 patients and five of 41 deteriorated by more than 13% with no change in FVC and mid-expiratory flow suggesting a variable improvement in FEV_1 [6].

Southern et al. in their meta-analysis at the two-month time point demonstrated a significant benefit with respect to percentage change in FVC from azithromycin, but no difference with respect to percentage change of FEV_1 [17]. In the study by Clement et al., though other parameters improved at the end of 12 months of treatment with AZT, the relative change in FEV_1 at month 12 did not differ significantly between the two groups [8]. Steinkamp et al. in a randomized double-blind, placebo-

Table 5
Average weight of patients at different time intervals.

Time of follow up	Group A (AZM 5 mg/kg/day)	Group B (AZM 15 mg/kg/day)	*p*
Baseline	17.48±13.33 (11.97–22.98)	16.43±9.54 (12.30–20.56)	0.75
3 months	17±17.42 (7.713–26.28)	18.3±11.57 (12.88–23.71)	0.53
6 months	21.09±18.80 (8.45–33.72)	19.09±10.94(11.73–26.44)	0.76
12 months	17.14±15.62 (8.11–26.16)	19.90±8.64 (14.10–25.71)	0.60
18 months	18.57±10.87 (8.51–28.62)	23.5±13.59 (12.13–34.86)	0.45
	p values comparing	*p* values comparing	
	Baseline with 6 month: 0.4	Baseline with 6 month: 0.37	
	Baseline with 12 months: 0.93	Baseline with 12 months: 0.19	
	Baseline with 18 months: 0.83	Baseline with 18 months: 0.12	

controlled trial concluded that once-weekly AZM ameliorated inflammatory reactions and improved quality of life. A decline of pulmonary function after cessation of intravenous antibiotics could not be prevented [18].

Heterogeneity in the treatment effect has been documented and depends on severity of disease, concomitant medications and genotype, however all patient may show some improvement in clinical outcomes [19]. In our study, we included all children irrespective of colonization with *Pseudomonas*, nutritional status and baseline FEV_1. The patients were assessed to be having moderately severe disease as indicated by average clinical scores of 54 and 53 in the two groups receiving AZM 5 mg/kg/day or 15 mg/kg/day respectively. Their percentage predicted FEV_1 was also 51% and 65% respectively. It is suggested that azithromycin may be altering *P. aeruginosa* biofilm formation and providing clinical benefit in cystic fibrosis patients [20]. In our study only 22 (46%) patients had chronic *Pseudomonas* infection. None of them were getting DNAse or inhaled tobramycin. These factors may explain lack of improvement in FEV_1. However, the randomization would have distributed the cases in both the limbs equally. It has been observed that azithromycin prevents decline in the FEV_1 as compared to placebo [5]. Since we did not have placebo group and data on the normal decline in FEV_1 in our study population, we may not be able to demonstrate any difference in the decline in FEV_1 as well as prevention of decline in FEV_1 in our study.

We documented high levels of IL-8 at enrolment and at completion of 6 months. There was no reduction in levels of IL-8 in both the groups at completion of 6 months of treatment. An anti-inflammatory mechanism of action has been suggested for macrolide antibiotics [21]. But earlier studies also have not shown statistically significant reductions in IL-8 [7] with AZM.

4.1. Limitations of study

Our study has certain limitations. The group of patients we enrolled included children with and those without colonization with *Pseudomonas*. Most studies demonstrated improvement in patients with colonization with *Pseudomonas*. This may be responsible for no significant improvement in FEV_1 from baseline to end of treatment. Other limitations of our study were we did not perform drug levels in sputum or blood nor did we estimate inflammatory markers (CRP, etc.) to document decrease in inflammation; these would have strengthened the results of our study. The reverse power calculation revealed a power of study as 70%, a larger study with adequate sample size with uniform characteristics of study subjects may be needed to confirm the results.

4.2. Summary and conclusions

The two doses of AZM (5 mg/kg/day or 15 mg/kg/day) did not differ in their effects on lung function, exacerbation rates and microbiology. The number of exacerbations increased significantly after 12 months of enrolment.

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